Congruity backcross (CBC) hybrids used to create three-species hybrids with *Phaseolus acutifolius* cytoplasm

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Introduction

Three-species CBC hybrids with *P. vulgaris* or *P. coccineus* cytoplasm have served to elucidate the importance of CBC as a means of gene transference between species. Traits such as drought tolerance, and seed color/size/patterning have been transferred with the use of such CBC hybrids (Mickelson, 1992; Petersen, 1993; Brown, 1994; Anderson and Ascher. 1995). *P. coccineus* cytoplasm (the ancestral species) frequently dominates the expression of most morphological and biochemical traits in early CBC generations, often preventing the detection of true hybrids (Brown, 1994). *P. vulgaris* cytoplasm hybrids have performed similarly, although its cytoplasm is not dominant to *P. coccineus*. Two-species *P. vulgaris* CBC individuals have exhibited less dominant cytoplasm, producing early and late CBC generations (*P. vulgaris x P. coccineus*) with cross-overs for diagnostic traits (Anderson and Ascher, 1995b). Stigma (introrse/extrorse) and cotyledon (hypogeal/epigeal) positions are strongly determined by the cytoplasmic parent, significantly skewing the hybrid distribution. Flower color and inflorescence architecture are also affected by cytoplasmic-genic interactions.

While two-species CBC hybrids with *P. acutifolius* cytoplasm exist, also dominated by the cytoplasmic parent, no three-species CBC pedigrees have been generated to date. This study was undertaken to generate such pedigrees and analyze whether the *P. acutifolius* cytoplasm was as strong an influence as either *P. vulgaris* or *P. coccineus*.

Materials and Methods

Two-species, CBC4 F_3 and F_4 hybrids derived from *P. acutifolius var. latifolius* (A_{10}; PI 406-622) x *P. vulgaris* 'Red Cloud' (V_1), with three doses of both parents ($A_{10}{}^3V_1{}^3$). were used as the cytoplasmic parent. $A_{10}{}^3V_1{}^3$ F_3 (♀) was crossed with the following male parents: 2WPC (a two-way, intraspecific *P. coccineus*; producing cross 94-32R) and 93-18-2 (a two-species CBC from crossing *P. vulgaris* 'Soldier' x 4WPC *P. coccineus*; producing cross 94-33R). $A_{10}{}^3V_1{}^3$ F_4 (♀) was crossed with the following male parents: 92-1-2 (a two-species CBC from crossing *P. vulgaris* 'Soldier' x 2WPC *P. coccineus*; producing cross 94-13R) and Pc x Pv (a two-species cross between *P. coccineus x P. vulgaris*; producing cross 94-19R). F_1 ($n = 1$-5) and F_2 ($n = 1$ or 20) progeny were screened for diagnostic morphological/biochemical traits (stigma and cotyledon positions. seed proteins, terminal leaf length/width ratios. primary inflorescence architecture, plant growth habit, and flower color) and fertility.

Results and Discussion

Pollen stainability remained low in the F_1 and F_2 generations (60-70% on average): female fertility (ability to) was <1 seed per pod, especially with crosses 94-19R and 94-13R. This low fertility hampered continued CBC with these genotypes. Thus, the most F_2 progeny ($n=20$) were obtained with 94-32R and 94-33R pedigrees.

In most cases, continued CBC produced parthenocarpic fruits with aborted embryos. Embryos aborted before the heart stage, eliminating the use of embryo rescue onto Murashige-Skoog medium.

Most crippled F_2, that either failed to complete germination or grew as cripples, had significant decreases in total seed protein production and/or a complete loss of phaseolin. 94-32R F_2 (n=20 plants) segregated as n=10 dead, n=5 cripples, n=5 normal plants; n=9 nongerminated, n=8 epigeal, n=1 intermediate, and n=2 hypogeal. 94-33R followed a similar pattern, although more F_2 had intermediate (n=4) or hypogeal (n=6) germination. Cross-overs for several traits did occur. For instance, there were n=2 94-33R F_2 that had hypogeal cotyledon positions, introrse stigmas, and white flowers. These segregated for plant growth habit (bush versus climbing). No F_2 in any pedigree displayed the *P. coccineus* phenotype of hypogeal germination and extrorse stigmas; only one genotype approached this (hypogeal/intermediate stigma) (Figure 1). Clearly, the *P. acutifolius* cytoplasm does not completely dominate in the progeny, allowing for specific ancestral *P. coccineus* cytoplasmic traits to become unlinked and recombined with *P. acutifolius/P. vulgaris*. If both *P. vulgaris* and *P. acutifolius* are derived from ancestral *P. coccineus*, with *P. acutifolius* being the most recently derived, then *P. acutifolius* cytoplasm would be more vulnerable to amelioration by *P. coccineus*. The unexpected linkage disequilibrium of strongly linked *P. coccineus* traits in the F_2 of 94-33R confirms that *P. acutifolius* is more recently derived, exhibiting only weak cytoplasmic dominance, but can readily recombine with *P. coccineus*.

References

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Figure 1. Cotyledon and stigma position for 94-33R-1 F_2 population, a three-species CBC pedigree with *P. acutifolius* cytoplasm.